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PATENT



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the **PATENT APPLICATION** of:

Lo et al.

**Application No.:** 09/872,063

**Confirmation No.:** 3772

**Filed:** June 1, 2001

**For:** NON-INVASIVE PRENATAL  
DIAGNOSIS

**Group:** 1655

**Examiner:** Goldberg, Jeanine Anne

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Commissioner for Patents  
Washington, D.C. 20231

Sir:

This Reply is responsive to the Official Action dated November 16, 2001 and is submitted in conjunction with an appropriate petition for extension of time, a Supplemental Information Disclosure Statement and a Section 132 Declaration. Please amend the following application as follows:

IN THE CLAIMS

Please amend claims 1, 6, 14, 16, 18, 19 and 31 to read as follows:

A!

1. (Amended) A detection method performed on a maternal serum or plasma sample from a pregnant female, which method comprises detecting the presence of a fetal

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nucleic acid in the sample by detecting nucleic acid which differs qualitatively or quantitatively from that of the maternal genome.

A1  
concl'd

A2

6. (Amended) The method according to claim 1, wherein the presence of a fetal nucleic acid sequence from the Y chromosome is detected.

A3

14. (Amended) The method according to claim 13, for Rhesus D genotyping a fetus in a Rhesus D negative mother.

A4

16. (Amended) The method according to claim 1, which comprises determining the concentration of a fetal nucleic acid sequence in the maternal serum or plasma.

A5

18. (Amended) The method according to claim 1, for the detection of a maternal or fetal disease condition in which the concentration of fetal DNA in the maternal serum or plasma is higher or lower than the concentration present in normal pregnancy.

19. (Amended) The method according to claim 1 for the detection of a fetal disease condition wherein the pattern of variation of fetal DNA concentration in the maternal serum or plasma at particular stages of gestation is different from that of normal pregnancy.

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**AQ** 31. (Amended) A method of non-invasive prenatal diagnosis for determining a fetal genetic condition comprising:

obtaining plasma or serum from a sample of a pregnant female's blood, detecting fetal nucleic acid within the serum or plasma and determining the presence or absence of one or more selected nucleic acid sequences in the detected fetal nucleic acid.

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Please cancel claims 24-30, without prejudice.

Please add the following new claims 33-36 as follows:

**AJ** --33. A detection method performed on a maternal serum or plasma sample from a pregnant female, which method comprises detecting the presence of a fetal nucleic acid in the sample by detecting nucleic acid which differs in sequence or amount from that of the maternal genome.

34. The method according to claim 16, wherein the fetal nucleic acid sequence is a chromosome 21 sequence.

35. The method according to claim 16, wherein an increase in the quantity of fetal DNA above a population mean indicates an increased risk of fetal aneuploidy.

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A2  
cont'd

36. A detection method performed on a maternal serum or plasma sample from a pregnant female, which method comprises (a) amplifying a fetal nucleic acid sequence or isolating fetal cells, from maternal plasma or serum and (b) detecting the presence of a nucleic acid of fetal origin in the amplified sequence or isolated cells. --

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#### **REMARKS**

All references in this response to paragraphs are to the paragraphs of the Publication of this application under Publication No. 2001/0051341 A1, which differ from those of the original typescript.

#### **Priority**

The acknowledgment of U.S. priority, 35 U.S.C. §371 and foreign priority status under the international convention has been noted with thanks. It is further noted that the parent patent application No. 09/380,696, filed on November 29, 1999, has issued to U.S. Patent No. 6,258,540.

#### **Claim Amendments**

Claim 1 has been amended and now specifies that the method proceeds "by detecting nucleic acid which differs qualitatively or quantitatively from that of the maternal genome". The amendment is for clarification purposes only and is in no way connected with prior art.

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Claim 14 has been amended, as suggested by the Examiner, and is now dependent upon Claim 13.

Claim 16 has been amended, as suggested by the Examiner, and is now dependent upon Claim 1.

Claims 18 and 19 have been amended to introduce mention of a disease condition and thus enable the term "normal" to be clarified as relating to the undiseased state. The amendment is no way connected with prior art.

Claims 24-30 have been deleted for reasons purely of expediency, as the subject matter is adequately covered by earlier claims. The deletion is in no way connected with prior art.

Claim 31 has been amended for clarification purposes only and now specifies a "genetic condition of the fetus".

New claims 33-36 are presented; no new matter has been added.

Basis for claim 34 can be found at paragraph [0020] of the application.

Basis for claim 35 can be found at Figure 1 and the accompanying text at paragraphs [0024] and [0073].

Basis for claim 36 can be found at paragraphs [0012] and [0161].

A marked-up copy of the claim amendments is attached.

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**Traversal Of Rejections**

Claims 1-32 have been rejected under 35 U.S.C. §112, first paragraph. The claims have been amended, but if and insofar as the examiner might deem the rejections of continuing applicability, applicants respectfully contest them for the following reasons.

The use of fetal nucleic acid analysis for a wide variety of diagnostic and other purposes is well known in the art. Conventional methods for obtaining fetal nucleic acid include invasive techniques which have the potential for harming the fetus itself. Applicants' discovery that fetal nucleic acid can be detected in maternal serum or plasma and disclosure of methods for detecting fetal nucleic acid in maternal serum or plasma as defined by independent claims 1 and 33 is a significant advance in the art. Using Applicants' teachings to detect fetal nucleic acid, much useful information is gained even at the earliest stages of pregnancy without any disturbance of the fetus whatsoever. While continued advances may improve detection, this does not detract from the fact that fetal nucleic acid can be detected using Applicants' claimed methods.

The instant application clearly teaches that large amounts of fetal nucleic acid are present in maternal serum or plasma from the first trimester and can be detected. The identification of such large amounts of fetal nucleic acid is, in itself, the solution to a significant technical problem, namely, how to obtain, non-invasively, analytically useful amounts of fetal nucleic acid for genetic analysis. The application, in addition to providing

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specific examples, also teaches the broad applicability of the invention to non-invasive prenatal genetic analysis see paragraphs [0160 and 0161].

The parent patent 6,258,540 claims in claim 1:

"A method for detecting a paternally inherited nucleic acid of fetal origin performed on a maternal serum or plasma sample from a pregnant female, which method comprises amplifying a paternally inherited nucleic acid from the serum or plasma sample and detecting the presence of a paternally inherited nucleic acid of fetal origin in the sample."

This is just one specific example which illustrates the utility of Applicants' claimed invention. Applicants seek in this continuing application to obtain claims that more fully reflect the generality of the invention. This is because the term "paternally inherited" does not cover the cases: (a) in which a gene is maternally inherited, yet the nucleic acid is not (in total) the same in the fetus as in the mother, and (b) in which the gene is altered spontaneously, for example, in the egg or sperm, i.e. by what appears to be chance or sporadic mutation. Also, it is not always necessary to amplify the nucleic acid in the sample in order to detect the fetal DNA.

Turning now to the specifics of the enablement rejections raised by the examiner under 35 U.S.C. §112, first paragraph, it is believed that many will be resolved by study of the enclosed Declaration of Professor Yuk-Min Dennis Lo, one of the inventors.

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At page 3, line 12 to page 4, line 5, the Examiner refers to certain papers of inventor Lo et al. which are not prior art, but which allegedly imply that the inventors had not conceived their invention as applicable to the first trimester of pregnancy, i.e. before the 15th week of gestation. Professor Lo deals with this point in section 4 of his Declaration, where he says:

"I now believe that these statements, made in refereed journals are over-cautious and that it was fully justified to take a wider view of the applicability of my work when the patent application was first prepared. Thus, I believe that those familiar with DNA amplification techniques would be able to achieve useful results from samples taken during the first trimester of pregnancy."

Professor Lo lists and explains the underlying evidence for this statement, by referring to no less than five different sources:

- (a) the patent application itself, Figures 4A to 4L and the associated description at printed paragraph [0152] where the SRY gene was consistently detected at between 7 and 14 weeks in 12 experiments;
- (b) a statement by another research group, Faas et al., in a 1998 paper, in which these authors refer to the very same data mentioned above as present in the patent application

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and acknowledge that these data showed detection of fetal DNA in maternal plasma in the first trimester of pregnancy;

- (c) a large scale experiment by another research group, Sekizawa et al., on 275 women in the first trimester of pregnancy, in which sex determination was carried out successfully;
- (d) another large scale experiment by another research group, Honda et al., on 75 women in the first trimester of pregnancy, in which sex determination was carried out successfully;
- (e) the detection by Amicucci et al. of a specific genetic mutation, involving a CTG repeat sequence, during the first trimester.

As Professor Lo states in relation to (d), "these detectable quantities of DNA could be utilized by those skilled in the art to detect any target sequence within the [fetal] DNA, just by the use of the appropriate primer sequence in relation to that target". It is immaterial whether the DNA detected is paternally inherited or not. See also, Lo's concluding statement in section 7 of his Declaration:

"I believe that the above examples show that when the competent person in the field has been taught, by the present invention, that the maternal plasma or serum contains sufficient fetal DNA in the high concentrations found, he would have no difficulty in carrying out fetal diagnosis to detect even small

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genetic defects, in any chromosomal locus, and even in the first trimester of pregnancy."

On page 5 of the Action, the claims are rejected on the ground that detecting non-paternally inherited fetal DNA in maternal plasma samples is unpredictable and requires amplification of the DNA. Applicants respectfully contest this rejection. The examples referred to above, in the Lo Declaration, of the practice of the present invention should be viewed as convincing and relevant to non-paternally inherited as well as paternally inherited DNA. The inherited source of the DNA cannot make any difference to the chromosomal concentration. It is irrelevant that the DNA is paternally inherited in the examples quoted above, since the critical teaching of the present invention is that ALL fetal DNA is greatly increased in concentration in the maternal plasma or serum as compared with maternal whole blood.

The rejection relating to amplification is also raised at page 6, lines 4-5, and 18 of the Action. At lines 4-5, the Examiner states "it is unpredictable that the fetal nucleic acids could be detected without first amplifying the fetal nucleic acids". We respectfully disagree with this statement. Whilst it may be preferable to use an amplification technique, this is not a limitation of the method of the invention. It is simply a matter of using a diagnostic method that gives a signal that is powerful enough to be detectable. The application clearly indicates that an enrichment or amplification step of some sort is preferable and usual, but it does not

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state that this is an essential part of the invention. Indeed the invention has been practiced without any amplification step, as shown by Poon et al., *The Lancet* 356, 1819-1820 (November 25, 2000) in which detection of fetal DNA was carried out on maternal plasma obtained at 12 & 15 (combined), 15 and 21 weeks using a probe specific for chromosome 21 to detect a chromosomal aneuploidy (Down's syndrome). This was achieved with a DNA probe attached to a fluorescent marker.

The Examiner links these rejections with an assertion that the specification does not provide any information as to the quantity of fetal nucleic acids present during the first trimester of pregnancy. It is true that there is no information in the specification about quantity, specifically related to weeks 7 to 14, but the specification does give information about the quantity in weeks 11 to 17 (and termination of the pregnancy at week 17 is still a possibility): see paragraph [0150] of the patent application, disclosing a mean fractional concentration of 3.4% in plasma and 0.13% in serum. Regardless of the exact numerical concentration, there is ample evidence that the invention works, as shown above and in the specification. Thus, Applicants respectfully contest the notion that there is a lack of information in the specification or that if there were any such lack relating to numerical concentrations, it would be in any way non-enabling for the carrying out of the method.

The Examiner, referring to a publication by the inventor, asserts on page 4, line 16 of the Action, that "Lo has not taught single gene disorders other than RhD..."

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In addition to providing the RhD example and discussing the general applicability of the invention to "fetal-derived paternally inherited polymorphisms/mutations or genes" paragraph [0160] of the patent application refers to a number of other conditions to which the invention may be applied, including sex-linked disorders, cystic fibrosis and hemoglobinopathies. This paragraph also refers to a 1994 paper by Lo that describes specific methods for the detection of cystic fibrosis. The term "single gene disorder" is a well recognized term of art and the mutations involved in known single gene disorders are usually well characterized. Given the information provided in the patent application and general knowledge of the skilled reader, the invention could be applied broadly to the detection of other single gene disorders. Indeed a number of workers have used the method of the invention to detect single gene disorders, including:

- (a) Saito et al., *The Lancet* 356, 1170 (2000) describe the diagnosis of fetal achondroplasia, a genetic defect which gives rise to short limbs. In nearly all cases, genetic defect is a single nucleotide change in the gene encoding human fibroblast growth factor receptor 3.
- (b) Amicucci et al. who detected a specific genetic mutation, involving a CTG repeat sequence, during the first trimester.

The Examiner noted that Amicucci et al. conceived that their results were appropriate only for paternally inherited mutations. This is the knowledge of cautious scientists

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operating under the conditions of peer reviewed literature. In fact there is no known reason why in principle a genetic mutation should not be detected equally easily in a maternally inherited gene; of course, such mutations, being essentially sporadic, are uncommon.

The Action contains a similar objection at page 7, lines 4 and 5 regarding simple point mutations, stating "it is unpredictable that a disease phenotype which occurs due to a single point mutation would be detectable". Single point mutations are examples of aberrations in the DNA sequence that may cause single gene disorders. The above mentioned paper by Saito et al. is an example of the use of the present invention to detect such a mutation.

Next, the official Action refers to Pertl et al. who conceived that the method of the invention is applicable to aneuploidies only if they are paternally inherited. In fact, the inventor Lo has demonstrated the applicability of the method to Down's syndrome which is not usually paternally inherited. This point is dealt with below.

As part of the enablement objection the Examiner states at page 6, lines 5 onwards of the Action, "The detection of a maternally inherited nucleic acid is unpredictable". Applicants respectfully traverse this objection. It would be obvious, given the abundance of fetal nucleic acid in maternal plasma or serum, that even a simple single nucleotide change would be detectable. It is immaterial how such a fetal sequence arises; it may be a paternally inherited sequence or it may arise as a result of a spontaneous mutation in either the egg or sperm. Thus the invention is not limited to the detection of paternally inherited fetal DNA.

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Whilst the application does not provide a specific example of the detection of a specific maternally inherited fetal nucleic acid sequence, it does provide examples of how the invention may be used to screen for a disease that is maternal in origin based on a quantitative assay. The additional chromosome 21 present in a Down's affected fetus, whilst not present in the genome of the mother, is usually derived from the egg and is thus maternally inherited. The application provides examples of screening for Down's syndrome, see Example 2, paragraph [0045], by measuring fetal DNA concentration using a paternally inherited marker; an elevated level being indicative of a Down's-affected fetus. An alternative strategy for the detection of Down's is presented in paragraph [0020]; this utilizes markers for chromosome 21 and another chromosome. These markers are not necessarily specific to a particular fetal specific DNA sequence; the intention is to amplify both maternal and fetal DNA and measure a difference in the concentration of the different chromosome markers. Obtaining diagnostically useful information regarding fetal aneuploidy is an important aspect of the invention.

Furthermore, the literature shows that at least two research groups have used the teaching of the patent application to detect a fetal aneuploidy using maternal serum or plasma, including:

- (a) Zhong et al., Prenatal Diagnosis 20, 795-798 (2000), who analyzed maternal plasma and observed "an almost two-fold elevation in the amount of fetal circulatory DNA

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in pregnancies with 47XY +21 fetuses when compared to pregnancies with normal male fetuses."

(b) Poon et al., The Lancet 356, 1819-1820 (November 25, 2000), where detection of fetal DNA was carried out on maternal plasma obtained at 12 & 15 (combined), 15 and 21 weeks using a probe specific for chromosome 21 to detect a chromosomal aneuploidy (Down's).

As part of the enablement rejection the Action refers specifically to claims 27 and 29-32, stating that the claims encompass "conditions and characteristics which are not enabled". Applicants respectfully traverse this objection; the method of the invention is potentially applicable to any genetic condition present in the fetus. Which particular genetic condition is being tested for is immaterial; providing the fetal nucleic acid differs qualitatively or quantitatively from that of the maternal genome, then the condition may be detected. Of course, any characteristic that is not truly of genetic origin, is excluded. What this invention achieves is to provide that a genetic change previously undetectable, because of the low concentration of nucleic acid in whole blood, will normally be detectable by starting from plasma or serum. Conditions that would have been undetectable regardless of the quality or quantity of the fetal DNA in the maternal blood will remain undetectable.

A specific enablement objection is raised on page 7 of the Action, at line 12 onwards, relating to claim 31. The objection states that the "absence of a sequence is not supported"

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and also that it "is unpredictable without amplification or enrichment". Applicants respectfully contest this objection as the application specifically shows that the absence of a particular sequence can be used reliably to determine the sex of the fetus. Moreover, amplification is not necessarily required as explained in detail above.

To summarize, the patent application clearly teaches Applicants' discovery that fetal nucleic acid can be detected maternal in serum or plasma from the first trimester and how to detect it. It also teaches examples of how this nucleic acid may be used to identify particular genes and enables the skilled man to broadly apply the method of the invention to the field of non-invasive prenatal genetic analysis.

Claims 1 and 5-32 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for allegedly failing to particularly point out and distinctly claim the invented subject matter.

It is believed that all these rejections have been answered by the above argument and/or claim amendment. Referring to the lettered paragraphs in the rejection:

- A) As pointed out and demonstrated above, amplification is not a necessary step.
- B, C and D) Dealt with by amendment, as noted above.
- E) Moot, following cancellation of the claim.
- F) The amendment to claim 31 answers the rejection made against claims 31 and 32; cancellation of the other claims renders the remainder of this rejection moot.

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With respect to the obvious type double patenting rejection, Applicants presently intend to submit a terminal disclaimer when the other issues of patentability are resolved.

With respect to Application 09/876,005, filed June 6, 2001, it is not commonly owned and Applicants' parent case which issued as U.S. Patent No. 6,258,540 is believed to be prior art in view of that patent's November 29, 1999 Section 102(e) date and September 11, 1998 PCT publication date. In particular, the patent specifically discloses the possibility of using fetal RNA at column 2, lines 6-18 and column 2, lines 26-41.

Reconsideration and allowance of the claims is respectfully requested.

Respectfully submitted,

Lo et al.

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